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Thiamin Requirement of Nile Tilapia, *Oreochromis niloticus*

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Abstract

Juvenile Nile tilapia, *Oreochromis niloticus*, were fed to apparent satiation twice daily with purified diets containing 0, 1.0, 2.0, 4.0, 8.0, and 16.0 mg/kg and 0, 2.0, 4.0, 8.0, 16.0, and 32.0 mg/kg of thiamin in separate 14- and 8-wk trials (Experiments 1 and 2, respectively). Fish fed the diet devoid of thiamin developed neurological disorders, anorexia, reduced growth, and feed efficiency and increased mortality (Experiment 2 only) within 4–6 and 8–10 wk for Experiments 2 and 1, respectively. Low red blood cell count (RBC) and hematocrit (Ht) were observed in fish fed the thiamin-deficient diet. Serum pyruvate was elevated in fish fed the thiamin unsupplemented diet. Serum lactate was not affected by dietary thiamin levels. Whole body protein was unaffected by dietary levels of thiamin. Body moisture and ash increased whereas body lipid decreased in fish fed the thiamin unsupplemented diets. None of these abnormalities were observed in fish fed the thiamin-supplemented diets. Using the response curves determined by PROC NLMIXED to estimate dietary thiamin levels required for various response variables, a dietary thiamin level of 3.5 mg/kg diet was adequate for optimum growth, feed intake and efficiency, survival, prevention of neurological symptoms, and maintaining normal levels of RBC, Ht, serum pyruvate, and proximate body composition.

Thiamin or vitamin B_1 is essential for growth and metabolism of all animals as well as of many plants and microorganisms (Oser 1979). Thiamin functions in all cells as the coenzyme thiamin pyrophosphate (TPP) necessary for several metabolic decarboxylation and transketolation reactions. It involves in the oxidative decarboxylation of α-keto acids, such as pyruvate to α-ketoglutarate, and in the transketolase reactions in the pentose phosphate pathway that are essential to provide pentose phosphate for nucleotide synthesis and dihydronicotinamide adenine dinucleotide phosphate (NADPH) for fatty acid synthesis (NRC 1993; Rindi 1996).

Thiamin has been demonstrated to be essential in the diets of fish and quantitative dietary thiamin requirements have been determined for several species including rainbow trout, *Salmo gairdneri* (McLaren et al. 1947; Morito et al. 1986); Pacific salmon, *Oncorhynchus* spp. (Halver 1972); common carp, *Cyprinus carpio* (Aoe et al. 1969); Jian carp, *C. carpio* var. Jian (Huang et al. 2010); channel catfish, *Ictalurus punctatus* (Murai and Andrews 1978); turbot, *Scophthalmus maximus* (Cowey et al. 1975); yellowtail, *Seriola quinqueradiata* (Shimino 1991); and red hybrid tilapia (Lim and Lea-Master 1991). The requirement values reported were 1–10, 10–15, 0.5, 1.02, 1.0, 2.60, 11.2, and 2.5 mg/kg diet for rainbow trout, Pacific

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salmon, common carp, Jian carp, channel catfish, turbot, yellowtail, and red hybrid tilapia, respectively. Deficiency signs observed for various fish species were anorexia, reduced weight gain (WG), nervous symptoms, convulsion, loss of equilibrium, muscle atrophy, abnormal coloration, fin congestion, subcutaneous hemorrhages, and high mortality (Woodbury 1943; Halver 1972; Morito et al. 1986; Lim and Lea-Master 1991; NRC 1993; Huang et al. 2010).

Erythrocyte or liver transketolase activity has been used as an indicator of thiamin status in terrestrial animals (Brin et al. 1960; Warnock 1970; Oser 1979) and fish (McCartney 1971; Cowey et al. 1975; Cowey 1976; Masumoto et al. 1987). TPP concentrations in erythrocytes and liver have been shown to be a more sensitive indicator of thiamin status of rainbow trout than erythrocyte or liver transketolase activity (Masumoto et al. 1987). Cowey et al. (1975) used erythrocyte transketolase activity as a parameter to estimate thiamin requirement of turbot. Serum or blood levels of lactate and pyruvate have also been used as indicators of thiamin deficiency in animal (Gubler 1961; Suberlich 1967) and fish (McCartney 1971; Morito et al. 1986).

As no information on thiamin requirement of Nile tilapia, *Oreochromis niloticus*, has been reported, the following two experiments were conducted to determine the requirement of juvenile Nile tilapia for dietary thiamin and its effect on growth performance, feed utilization efficiency, hematology, and serum pyruvate. As serum pyruvate and lactate have been reported as sensitive indicators for thiamin deficiency, serum concentrations of these parameters of fish in Experiment 2 were evaluated after 4, 6, and 8 wk of feeding. Whole body proximate composition at the end of Week 8 was also determined for fish in Experiment 2.

Materials and Methods

Experimental Fish, Diets, and Feeding

Nile tilapia, *O. niloticus*, spawned and reared at our laboratory to juveniles on commercial fry diets were acclimated to the experimental basal diet for 2 wk prior to stocking. During this period they were fed the basal diet (without added thiamin) twice daily to apparent satiation. At the end of the acclimation period, fish with an average weight of 5.30 ± 0.10 g were randomly selected and stocked in 24, 110-L aquaria at a rate of 40 fish/aquarium (Experiment 1). In Experiment 2, fish averaging 3.62 ± 0.15 g were randomly stocked at a rate of 35 fish in each of the 24, 57-L aquaria. Aquaria were supplied with flow-through dechlorinated, heated city water at an initial rate of about 0.6 L/min and increased gradually to about 1.0 L/min by the sixth week. Water was continuously aerated using air stones. In each experiment, water temperature and dissolved oxygen (DO) in four randomly chosen aquaria were measured once every other day in the morning, using an YSI model 58 Oxygen Meter (Yellow Spring Instrument Co., Inc., Yellow Spring, OH, USA).[2] During the trial, water temperatures and DO averaged 26.2 ± 0.2 C and 5.84 ± 0.10 mg/L, and 29.7 ± 0.2 C and 5.50 ± 0.10 mg/L for Experiments 1 and 2, respectively. Photoperiod was maintained at a 12:12 h light : dark schedule.

The basal semipurified diets used in both experiments are presented in Table 1. Diets for Experiments 1 and 2 were formulated to contain approximately 40% protein and 3640 kcal of digestible energy (DE)/kg, and 34% protein and 3200 kcal of DE/kg based on the feedstuff values reported in NRC (1993) for channel catfish, respectively. Since an earlier study at our laboratory showed that juvenile Nile tilapia (6 g) performed equally well on isocaloric diets (3800 kcal DE/kg) containing 32 or 40% protein, a diet with 34% protein and 3200 kcal DE/kg was used in Experiment 2. The dry ingredients of each diet were thoroughly mixed in a Hobart mixer (Hobart Corporation, Troy, ID, USA) before the oil was added. After the oil was diffused into the mix, approximately 250 mL of deionized water/kg of diet was added. The moist mixture was extruded through a 3-mm diameter die in a Hobart meat grinder.

[2] Use of trade name or commercial products is solely for purpose of providing specific information and does not imply endorsement by the USDA.

TABLE 1. *Composition and estimated nutrient contents of basal diets used in Experiments 1 and 2.*

Ingredient	Percent (%) in diet	
	Experiment 1	Experiment 2
Casein (vitamin-free)	35.5	32.0
Gelatin	11.2	8.0
Corn starch	34.3	33.0
Corn oil	4.0	3.0
Cod liver oil	3.0	3.0
Carboxymethyl cellulose	3.0	3.0
Mineral mix[1]	4.0	4.0
Vitamin mix (thiamin-free)[2]	1.0	1.0
Ethoxyquin	0.02	0.02
Celufil	3.98	12.98
Estimated nutrient (air dry weight)		
Protein (%)	40.0	34.0
Lipid (%)	7.2	6.2
DE (kcal/kg diet)	3,640	3,200

[1]Mineral mix: Williams and Briggs (1963) salt mixture supplemented in mg/kg diet with cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), 4; aluminum potassium sulfate dodecahydrate [$AlK(SO_4)_2 \cdot 12H_2O$], 5.18 and sodium selenite ($Na\ SeO_3$),0.32.

[2]Thiamin-free vitamin mix, diluted in cellulose, provided the following in mg/kg diet: retinyl acetate (500,000 IU/g), 8; cholecalciferol (1,000,000 IU/g), 2; menadione sodium bisulfate, 10; α-tocopheryl acetate (250 IU/g), 200; riboflavin, 20; pyridoxine hydrochloride (82.3%), 24.3; panthothenic acid (91.9%), 21.8; nicotinic acid, 150; folic acid, 5; cyanocobalamin (0.1%), 20; biotin, 2; myo-inositol, 200; choline chloride (74.6%), 2,681; L-ascorbyl-2-polyphosphate (45% vitamin C activity), 222.2.

The resulting moist pellets were air-dried at room temperature to a moisture content of about 10%. Pellets were ground into small pieces, sieved to obtain approximate sizes, and stored frozen in plastic bags at −20 C until fed.

In Experiment 1, six diets supplemented with thiamin hydrochloride at 0, 1, 2, 4, 8, and 16 mg/kg diet were fed to juvenile Nile tilapia for 14 wk. Because the results of Experiment 1 indicated that tilapia averaging 5.30 g in weight exhibited thiamin deficiency signs within 6 wk and the thiamin requirement appeared to be higher than 1 mg/kg diet, and since smaller fish (3.62 g) were used in Experiment 2, fish were fed for 8 wk only with diets supplemented with higher levels of thiamin (0, 2, 4, 8, 16, and 32 mg/kg diet). In both experiments, fish in four aquaria were randomly assigned to each of the experimental diets and were fed twice daily (0730–0830 and 1500–1600 h) to apparent satiation. The amount of diet consumed was recorded daily by calculating the differences in weight of diets prior to the first and after the last feeding. Once a week, aquaria were scrubbed and accumulated waste was siphoned. On cleaning days (once weekly), fish were fed only in the afternoon. Fish were not fed on sampling days.

Fish Sampling and Sample Collection

In Experiment 1, fish in all aquaria were group-weighed and counted biweekly for estimation of WG and survival. At the end of the feeding period (Week 14), three fish were randomly collected from each aquarium and anesthetized with tricaine methanesulfonate (MS-222; Argent Chemical Laboratories, Redmond, WA, USA) at 150 mg/L. Blood samples were collected from the caudal vasculature with dried heparinized (100 U) tuberculin syringes for hematological assays. An additional three fish/aquarium were also bled using non-heparinized tuberculin syringes and blood allowed to clot overnight at 4 C. Serum samples were collected following centrifugation at

TABLE 2. *Mean final weight gain, feed intake, feed efficiency ratio (FER) and survival of Nile tilapia fed diets containing various levels of thiamin for 14 wk (Experiment 1).*[1]

Dietary level of thiamin (mg/kg)	Weight gain (g/fish)	Dry matter feed intake (g/fish)	FER[2]	Survival (%)
0	46.5[c]	47.6[b]	0.97[b]	89.9
1.0	73.4[ab]	63.9[a]	1.15[a]	90.0
2.0	76.3[a]	64.6[a]	1.18[a]	95.6
4.0	70.6[ab]	62.3[a]	1.13[a]	95.5
8.0	66.6[b]	59.2[a]	1.13[a]	93.2
16.0	64.9[b]	58.8[a]	1.10[a]	95.0
Pooled SEM	3.58	2.38	0.03	2.42
Requirement estimate (mg thiamin/kg diet)	0.92	0.89	0.87	NQ

NQ = non-quadratic.

[1]Values are means of four replicates per treatment. Means in the same column with different superscripts are significantly different at $P < 0.05$.

[2]FER = weight gain (g)/dry feed fed (g).

1000*g* for 5 min and stored at −80 C for subsequent assays for serum pyruvate.

In Experiment 2, fish were group-weighed and counted at the end of Weeks 4, 6, and 8 for determination of WG and survival. Two fish/aquarium at the end of Weeks 4 and 6, and three fish/aquarium at the end Week 8 were randomly sampled, bled, serum collected, and stored at −80 C for measurement of serum pyruvate and lactate. Three additional fish/aquarium were also bled at the end of Week 8 for hematological assays. Blood and serum collections were done as described for Experiment 1. The six fish/aquarium that were bled at Week 8 for hematological and serological assays were pooled, stored at −20 C for subsequent determination of whole body proximate composition.

Hematological Assays

Hematocrit (Ht), red blood cell count (RBC), white blood cell count (WBC), and hemoglobin (Hb) were determined for blood samples collected at the end of Weeks 14 (Experiment 1) and 8 (Experiment 2) following the method of Brown (1988). Ht of each fish was determined in duplicate using the microhematocrit method. RBC and WBC were performed in duplicate for each sample by diluting (1:10,000) whole blood in phosphate buffer saline solution and enumerating in a Spencer Bright Line hemacytometer (Huasser Scientific, Horsham, PA, USA). Hb was determined using a cyanomethemoglobin method (Sigma Chemical Co., St. Louis, MO, USA). Hb was analyzed using a kit from Pointe Scientific, Inc. (Canton, MI, USA), and values were adjusted by cyanomethemoglobin correction factor as described by Larsen (1964) for channel catfish.

Serum Pyruvate and Lactate

Serum pyruvate concentrations were determined using an enzymatic pyruvate assay kit (Biovision Research Products, Mountain View, CA, USA). In this assay, pyruvate is oxidized by pyruvate oxidase via enzyme reactions to generate color (with an absorbance maximum at 570 nm) that is proportional to the pyruvate concentration. After 30-min incubation at room temperature, the absorbance of the samples was read at 560 nm. Serum pyruvate concentrations were calculated using the standard curve developed by using the pyruvate standard. Sera from fish in Experiment 2 were also analyzed for lactate using an enzymatic lactate kit (Sigma-Aldrich Corp., St Louis, MO, USA). In this procedure, lactic acid is converted to pyruvate and hydrogen peroxide (H_2O_2) by lactate oxidase. In the presence of the H_2O_2 formed, peroxidase catalyzes the oxidative condensation of chromogen precursors to produce a colored dye (with an absorption maximum at 540 nm) that is directly proportional to the lactate concentration in the sample. After 5- to 10-min incubation at room temperature, the absorbance of the standard and test sera were read at 540 nm. One determination of pyruvate or lactate was made for each of the serum samples.

Proximate Body Composition

The frozen fish samples were thawed in refrigerator overnight at 4 C prior to scale removal. After descaling, the pooled sample of fish from each aquarium were finely ground in a Hobart meat grinder and analyzed in duplicate for proximate composition following the standard methods (AOAC 1990). Moisture content was determined by drying samples in an oven at 105 C until constant weight was reached. Samples used for dry matter were digested with nitric acid and incinerated in a muffle furnace at 600 C overnight for measurement of ash contents. Protein was measured by combustion method using a FP-2000 Nitrogen Analyzer (Leco Corp., St. Joseph, MI, USA). Lipid content was determined by petroleum ether extraction using a Soxtec System (2055 Soxtec Avanti; Foss Tecator, Höganäs, Sweden).

Statistical Analysis

The concentration of dietary thiamin was the fixed effect in this study. Data were analyzed using PROC MIXED (SAS Inst., Inc., Cary, NC, USA) for a one-way analysis of variance (ANOVA). For response variables that were

significantly different, pairwise comparisons between treatment means were made using the Duncan multiple range test. When the quadratic term in the model was statistically significant, the relationship between thiamin level and measured parameter was further evaluated using PROC NLMIXED (Robbins et al. 2006) to determine the response curve and estimate the optimum thiamin requirement level in relationship to the measured parameter. A significance level of $P = 0.05$ was used for all statistical analyses.

Results

Experiment 1

After approximately 6 wk into the feeding experiment, fish fed the thiamin unsupplemented diet developed nervous symptoms (easily excitable) and by the eighth week, feeding response began to decrease (anorexia). By the end of Week 10, the average WG of fish in this treatment became significantly lower than those of fish in the other treatments. These signs were observed for the groups fed thiamin supplemental diets. Mean WG, dry matter feed intake (FI), feed efficiency ratio (FER) and survival at the end of Week 14 of fish fed various dietary thiamin levels are presented in Table 1. Significantly lowest WG, FI, and FER were obtained in fish fed the thiamin unsupplemented diet. WG of fish fed diets supplemented with 8 and 16 mg thiamin/kg were significantly lower than that of the diet with 2 mg added thiamin/kg but did not differ from those of the groups fed 1- and 4-mg thiamin diets. There were no differences among FI and FER of the groups fed the thiamin-supplemented diets. Survival was not affected by supplementation of dietary thiamin. Using PROC NLMIXED to determine estimates of the response curves, the estimated dietary thiamin concentrations for optimum WG, FI, and FER were 0.92, 0.89, and 0.87 mg/kg diet, respectively. The regression of survival on dietary levels of thiamin was linear (not quadratic).

Ht, WBC, and Hb did not differ among treatments (Table 3). The value of RBC of fish fed the 2-mg thiamin diet was significantly higher than those of fish fed diets supplemented with 0, 1, 4, and 16 mg thiamin/kg but did not differ from that of fish fed the 8-mg thiamin diet. Serum pyruvate concentration was significantly elevated in fish fed the thiamin unsupplemented diet. The values of this variable did not differ in fish fed diets with added thiamin. The estimated dietary thiamin levels determined for RBC and serum pyruvate using the response curves determined by PROC NLMIXED were 2.46 and 2.55 mg thiamin/kg diet, respectively. For WBC, Ht, and Hb, the responses to increasing dietary levels of thiamin was non-quadratic.

TABLE 3. *Mean red blood cell count (RBC), white blood cell count (WBC), hematocrit (Ht), hemoglobin (Hb), and serum pyruvate of Nile tilapia fed diets containing various levels of thiamin for 14 wk (Experiment 1).*[1]

Dietary level of thiamin (mg/kg)	RBC ($\times 10^6$)	WBC ($\times 10^5$)	Ht (%)	Hb (g/dL)	Serum pyruvate (mg/dL)
0	2.19[c]	3.62	25.71	10.33	0.76[a]
1.0	2.15[c]	2.98	27.78	11.09	0.38[b]
2.0	2.65[a]	3.33	27.28	11.16	0.29[b]
4.0	2.32[bc]	3.53	28.83	9.81	0.19[b]
8.0	2.56[ab]	3.57	26.21	10.25	0.22[b]
16.0	2.32[bc]	3.48	28.33	11.30	0.21[b]
Pooled SEM	0.01	0.36	1.05	0.77	0.08
Requirement estimate (mg thiamin/kg diet)	2.46	NQ	NO	NQ	2.55

NQ = non-quadratic.

[1]Values are means of two determinations per fish, three fish tank and four tanks per treatment for RBC, WBC, and Ht, and one determination per fish, three fish per tank and four tanks per treatment for Hb and serum pyruvate. Means in the same column with different superscripts are significantly different at $P < 0.05$.

Experiment 2

Neurological symptoms and anorexia in fish fed the thiamin unsupplemented diet became apparent during the fourth week into the trial. By the end of Week 6, the WG and feed consumption of fish in this treatment became significantly lower than those of fish in the other treatments. During this period, fish were lethargic and had difficulty in maintaining the position when passed through the water current created by the airstone. Mortality began to occur during Week 6 in fish fed the thiamin unsupplemented diet. No deficiency signs were observed for the groups fed thiamin supplemental diets. Mean WG, FI, FER, and survival at the end of Week 8 were significantly lowest in fish fed the thiamin unsupplemented diet (Table 4). There were no differences among WG, FER, and survival of fish fed various dietary thiamin levels. Dry matter FI of fish fed the 32-mg thiamin diet was significantly higher than that of the group fed the 2-mg thiamin diet. These values, however, did not differ from those of fish fed diets supplemented with 4, 8, and 16 mg thiamin/kg. Using the response curves determined by PROC NLMIXED, supplemental thiamin levels of 3.31, 3.17, 3.39, and 1.80 mg/kg diet were sufficient for optimum WG, FI, FER, and survival, respectively.

RBC, WBC, and Hb were not affected by supplementation of dietary thiamin (Table 5). However, RBC and WBC values tended to be lower in fish fed the thiamin unsupplemented diet. Ht significantly decreased in fish fed the thiamin unsupplemented diet but did not differ among fish receiving other treatments. The optimum level of supplemental thiamin estimated for this variable using PROC NLMIXED was 1.80 mg/kg diet.

Mean serum pyruvate and lactate at Weeks 4, 6, and 8 of fish fed diets containing various levels of thiamin are presented in Table 6. Serum pyruvate concentrations at Weeks 4, 6, and 8 were significantly higher in fish fed the diet without thiamin supplementation as compared to those fed other diets. The values of this parameter at various time periods did not differ among fish fed diets containing supplemental thiamin. The estimated dietary thiamin levels required to prevent elevated levels of serum pyruvate determined by PROC NLMIXED were 3.18, 3.48, and 2.21 at Weeks 4, 6, and 8, respectively. There were no significant differences among

TABLE 4. *Mean final weight gain, feed intake, feed efficiency ratio (FER), and survival of Nile tilapia fed diets containing various levels of thiamin for 8 wk (Experiment 2).*[1]

Dietary level of thiamin (mg/kg)	Weight gain (g/fish)	Dry matter feed intake (g/fish)	FER[2]	Survival (%)
0	25.7[b]	24.0[c]	1.07[b]	89.0[b]
2.0	40.5[a]	33.9[b]	1.19[a]	98.6[a]
4.0	42.7[a]	35.2[ab]	1.21[a]	100[a]
8.0	43.8[a]	35.9[ab]	1.22[a]	95.1[a]
16.0	42.7[a]	34.9[ab]	1.23[a]	98.6[a]
32.0	43.7[a]	36.2[a]	1.20[a]	98.9[a]
Pooled SEM	1.07	0.6	0.02	1.78
Requirement estimate (mg thiamin/kg diet)	3.31	3.17	3.39	1.80

[1]Values are means of four replicates per treatment. Means in the same column with different superscripts are significantly different at $P < 0.05$.

[2]FER = weight gain (g)/dry feed fed (g).

TABLE 5. *Mean red blood cell count (RBC), white blood cell count (WBC), hematocrit (Ht), and hemoglobin (Hb) of Nile tilapia fed diets containing various levels of thiamin for 8 wk (Experiment 2).*[1]

Dietary level of thiamin (mg/kg)	RBC ($\times 10^6$/μL)	WBC ($\times 10^5$/μL)	Ht (%)	Hb (g/dL)
0	2.05	2.19	29.55[b]	9.97
2.0	2.27	2.49	34.53[a]	10.27
4.0	2.45	2.30	33.63[a]	10.27
8.0	2.33	2.50	34.30[a]	9.94
16.0	2.51	2.24	32.90[a]	10.39
32.0	2.35	2.56	34.93[a]	10.46
Pooled SEM	0.11	0.18	1.06	0.28
Requirement estimate (mg thiamin/kg diet)	NQ	NQ	1.80	NQ

NQ = non-quadratic.

[1]Values are means of two determinations per fish, three fish tank and four tanks per treatment for RBC, WBC, and Ht, and one determination per fish, three fish per tank and four tanks per treatment for Hb. Means in the same column with different superscripts are significantly different at $P < 0.05$.

TABLE 6. *Mean serum pyruvate and lactate at Weeks 4, 6, and 8 of Nile tilapia fed diets containing various levels of thiamin for 8 wk (Experiment 2).*[1]

Dietary level of thiamin (mg/kg)	Pyruvate (mg/dL) at Week			Lactate (mg/dL) at Week		
	4	6	8	4	6	8
0	0.90[a]	1.03[a]	0.96[a]	14.10	14.42	9.34
2.0	0.67[b]	0.66[b]	0.37[b]	19.57	14.39	11.18
4.0	0.67[b]	0.62[b]	0.38[b]	13.90	16.49	11.51
8.0	0.67[b]	0.54[b]	0.40[b]	14.30	10.18	10.40
16.0	0.58[b]	0.58[b]	0.33[b]	14.33	13.88	12.31
32.0	0.62[b]	0.55[b]	0.35[b]	15.53	11.58	11.21
Pooled SEM	0.04	0.07	0.07	1.82	1.73	1.61
Requirement estimate (mg thiamin/kg diet)	3.18	3.48	2.21	NQ	NQ	NQ

NQ = non-quadratic.

[1]Values are means of one determination per fish, two fish per tank and four tanks per treatment for Weeks 4, 6, and 1 determination per fish, three fish per tank and four tanks per treatment for Week 8. Means in the same column with different superscripts are significantly different at $P < 0.05$.

TABLE 7. *Mean whole body proximate composition of Nile tilapia fed diets containing various levels of thiamin for 8 wk (Experiment 2).*[1]

Dietary level of thiamin (mg/kg)	Moisture (%)	Percent (%) wet weight basis		
		Protein	Lipid	Ash
0	72.87[a]	16.94	6.01[b]	3.78[a]
2.0	70.88[b]	16.37	8.87[a]	3.19[b]
4.0	70.44[b]	16.77	8.74[a]	3.30[b]
8.0	70.61[b]	16.50	8.69[a]	3.27[b]
16.0	70.98[b]	16.78	8.22[a]	3.26[b]
32.0	70.65[b]	16.55	8.62[a]	2.24[b]
Pooled SEM	0.38	0.13	0.30	0.02
Requirement estimate (mg thiamin/kg diet)	2.91	NQ	1.65	1.85

NQ = non-quadratic.

[1]Values are means of two determinations of pooled samples of three fish per tank and four tanks per treatment. Means in the same column with different superscripts are significantly different at $P < 0.05$.

serum lactate of fish receiving various dietary treatments.

Whole body protein was not influenced by supplemental levels of dietary thiamin (Table 7). Whole body moisture and ash contents significantly increased, whereas body lipid significantly decreased in fish fed the diet without thiamin supplementation. The values of these parameters did not significantly differ among fish fed diets supplemented with 2 to 32 mg thiamin/kg. Based on the response curves obtained by PROC NLMIXED, dietary thiamin levels of 2.91, 1.65, and 1.85 were sufficient to maintain optimum levels of whole body moisture, lipid, and ash, respectively.

Discussion

Published information appears to suggest that the responses of fish to thiamin deficiency vary among species, fish size, and dietary levels of carbohydrate. In this study, juvenile Nile tilapia fed the diet without thiamin supplementation developed nervous symptoms (easily excited when approached), reduced feed consumption (anorexia), instability, lethargy, poor growth and feed efficiency, and high mortality (Experiment 2 only). These signs are similar to those reported for rainbow trout (Woodbury 1943; McLaren et al. 1947; Aoe et al. 1967; Morito et al. 1986; Matsumoto et al. 1987), Pacific salmon (Halver 1972), turbot (Cowey et al.

1975), eel (Hashimoto et al. 1970), and red hybrid tilapia (Lim and LeaMaster 1991) fed thiamin-deficient diets. In contrast, fading of body color observed in common carp (Aoe et al. 1969) and red hybrid tilapia (Lim and LeaMaster 1991), dark skin pigmentation in channel catfish (Murai and Andrews 1978) and rainbow trout (Morito et al. 1986; Matsumoto et al. 1987), and fin congestion in eel, *Anguilla japonica* (Matsumoto et al. 1987), Jian carp (Huang et al. 2010), and rainbow trout (Morito et al. 1986) were not observed in our study. Murai and Andrews (1978) reported the absence of neurological disorders in channel catfish fed the thiamin-deficient diet for 20 wk. This symptom was likewise not detected in Jian carp fed thiamin-deficient diet for 60 d (Huang et al. 2010). Aoe et al. (1969) obtained nervous disorders in common carp only when fish were fed a diet supplemented with pyrithiamin, a thiamin antagonist. Huang et al. (2007) did not observe any obvious gross thiamin deficiency symptoms in juvenile grouper, fed graded levels of dietary thiamin (0.08–12.37 mg/kg) for 10 wk.

Neurological symptoms in Nile tilapia fed the thiamin-deficient diet occurred at Week 6 in Experiment 1 (average initial weight of 5.30 g) and at Week 4 in Experiment 2 (average initial weight of 3.62 g). This would indicate that younger or smaller fish that have a faster growth rate are more sensitive to thiamin deficiency. Morito et al. (1986) obtained neurological disorders after 4.5 and 11 wk in rainbow trout with average initial weights of 0.5 and 24.1 g, respectively. Red hybrid tilapia (1.76 g average initial weight) fed the thiamin unsupplemented diet developed neurological disorders at Week 6 (Lim and LeaMaster 1991).

Because thiamin functions as a coenzyme in carbohydrate metabolism, higher carbohydrate (corn starch) to protein ratio (0.97) in the basal diet used in Experiment 2 relative to that in Experiment 1 (0.87) may have also contributed to the increased thiamin requirement, thus accelerating the adverse effect of thiamin deficiency. Aoe et al. (1967) obtained no thiamin deficiency signs after 16 wk of feeding juvenile common carp test diets high in protein and low in carbohydrate. When these diets were replaced by the carbohydrate-rich diets, thiamin deficiency symptoms were demonstrated after 11 wk of feeding (Aoe et al. 1969).

Hematological variables, particularly RBC and Ht, determined at the end of Weeks 14 and 8 (Experiments 1 and 2, respectively) are inconsistent. In Experiment 1, fish fed diets without or with 1 mg thiamin/kg recorded significantly lower RBC than the groups fed 2 or 8 mg dietary thiamin. In Experiment 2, the value of this variable did not significantly differ among treatments, but fish fed the diet without added thiamin had lower RBC. This appears to indicate that 1 mg thiamin/kg diet was insufficient for juvenile Nile tilapia to maintain normal level of erythrocyte. However, significantly lower RBC values in fish fed 4- and 16-mg thiamin diets relative to that in fed the 2-mg thiamin diet cannot be explained. Nevertheless, based on the response curve determined using PROC NLMIXED, a supplemental thiamin level of 2.46 mg/kg diet was adequate to prevent decreased RBC. The significantly lower Ht in fish fed the thiamin unsupplemented diet obtained in Experiment 2 is in agreement with that reported by Lim and LeaMaster (1991) for juvenile red hybrid tilapia fed the thiamin-deficient diet. In contrast, Morito et al. (1986) obtained no significant differences in the Ht and Hb among rainbow trout after 12 wk of feeding diets without or with 15 mg thiamin/kg. The reduced RBC and Ht may be the result of insufficient nutrient intake for normal hematopoiesis due to decreased feed consumption after the onset of thiamin deficiency.

Accumulation of blood lactate and pyruvate in thiamin deficient animal has been reported (Gubler 1961; Sauberlich 1967). In this study, analyses of sera of fish at the end of Week 14 (Experiment 1) and Weeks 4, 6, and 8 (Experiment 2) showed a significant increase in the serum pyruvate levels in the groups fed thiamin unsupplemented diets. Serum lactate concentrations (Experiment 2), however, were not affected by dietary treatments. Morito et al. (1986) obtained a significant increase in the serum pyruvate levels in rainbow trout after 12 wk of feeding the thiamin-deficient diet. They

also recorded a numerical increase in levels of plasma lactate but no significant differences were detected among trout fed thiamin deficient or replete diets. Our data showed that serum pyruvate is a sensitive indicator of thiamin status in Nile tilapia as significant increase in the value of this parameter can be detected as early as 4 wk. In trout, significant differences in serum pyruvate were obtained at the end of Weeks 12 but not after 4 wk of feeding the experimental diets (Morito et al. 1986).

Decreased whole body lipid and increased body moisture and ash were observed in tilapia fed the diet without thiamin supplementation. Similar findings have been demonstrated in Jian carp fed the thiamin-deficient diet (Huang et al. 2010). These workers also obtained significantly increased body protein by dietary supplementation of thiamin. In contrast, we found that body protein content was not affected by supplemental levels of dietary thiamin. For abalone, Zhu et al. (2002) showed that dietary levels of thiamin had no adverse effects on their body proximate composition.

Data from this study showed that the thiamin requirement of juvenile Nile tilapia varies depending on the parameter evaluated. However, a dietary thiamin level of 3.5 mg/kg diet was adequate for optimum growth, FI and efficiency and survival and maintaining normal levels of RBC, Ht, serum pyruvate, and proximate body composition. This requirement value is higher than that of common carp, 0.5 mg/kg diet (Aoe et al. 1969), within the range reported for rainbow trout, 1–10 mg/kg diet (McLaren et al. 1947; Morito et al. 1986), higher than those reported for Jian carp, 1.02 mg/kg diet (Huang et al. 2010), channel catfish, 1.0 mg/kg diet (Murai and Andrews 1978), turbot, 2.6 mg/kg diet (Cowey et al. 1975), and red hybrid tilapia, 2.5 mg/kg diet (Lim and LeaMaster 1991) but lower than those reported for Pacific salmon, 10–15 mg/kg diet (Halver 1972) and yellowtail, 11.2 mg/kg diet (Shimino 1991).

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